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**Breeding information for NIDA OTTC generated transgenic rats**

1) The NIDA OTTC rats generated using random integration were bred as hemizygotes (or "het" or "carrier") x Long Evan from Charles River Laboratories (catalog # 006). For targeted constructs, the were bred at least 6 generations as true heterozygotes then pair for homozygous breeding as needed.
    a. It is important to use the same background if you want to be able to compare your results with what has already been published or observed. Please be aware that the LE from CRL would be quite different than the ones from other sources as the colonies have been separated and evolved differently for over the years now (potentially lots of generations). We have been crossing them with LE for at least 5 generations in our facility.
    b. If you are getting the animals through the RRRC, please confirm with them the source of their WT carriers.
2) We usually use a males hemizygote x female LE but females hemizygote x male LE works as well (although in our experience, the latter results in lower rates of breeding).
3) In our facility, the average litter is 9-10 pups every 2 months per breeding pair. Half of the pups are hemizygotes (and half WT). Therefore if, for example, 10 pairs are set up, you can expect a production of 25 experimental animals (hemizygotes, males and females) per month on average.
4) We target the wean the litters at 21 days but can do it between 19 and 24 days of age, to take in account week-ends and holidays.
5) We set up the breeders when they are between 2 and 4 months of age to keep the breeding colony at its optimum production. The first litter appears usually 30-45 days after the setting up of the breeding pair.
    a. Younger than 2 months old or older than 5 months old can result in delayed breeding.
    b. We suggest to make sure that the female is not too much older than the male: an older and bigger female can get intimidating to the male and he will then not breed. Having a female younger than the male does not appear to affect breeding.
6) Breeders are retired (stop breeding and euthanize) following the general rules below:
    a. Breeders become older than 10 months old.
    b. The pair does not have a litter in 3 months.
    c. The most recent litters have been getting smaller and smaller (for example if you see a pair having normally 10 pups per litter getting only 7 then only 5 then 3, we recommend retirement).
    d. The female had 7 litters.
7) We generally refresh the breeders every 5-6 months or so (unless the breeders had to be retired earlier as indicated above, in which case we replace them immediately with a new pair): after 5-6 months of breeding a particular pair, we set up a new one (with young new breeders) that will eventually replace the old one. We keep both pairs breeding in parallel until the younger pair has a

successful litter (surviving litter with at least one confirmed hemizygote) and only then it is safe to retire and euthanize the old pair. With that schedule, you can use the first 2 or 3 litters from a pair for experimental use and keep some animals for breeding from the 3rd of 4th litter and by the time you get these breeding, the parents would have had 2 or 3 more litters before they retire.

8) You can set up males in harems with 2 females each: you will need to separate the first pregnant female to avoid to have 2 litters in the same cage. From that point on, the male will be switched back and forth between the 2 cages (each one having a female):
    a. On a regular basis, you can switch that male every 2-3 weeks for example with the exception below
        i. Do not re-introduce the male with a female who is pregnant or who has a litter: the male might not "recognize" his pups and kill them.
9) Depending on which size of cages you are using, you might need to separate the male at birth, leaving only the female + pups in the cage (to avoid overcrowding the cage). If that is the case, re-introduce the male as soon as the litter is weaned. Females with pups can become very protective and aggressive and if that happens, the male should be separated as well if he presents signs of being bitten, for example.
10) Always keep a few young hemizygotes at all time (preferably males) for backup, to use as breeders if you need to (breeder that dies or stopped producing unexpectedly). We recommend setting aside ¼ of your total number of breeding pairs from the first litters you get. As new litters arrive, keep some of the younger pups at which point you can use the previous backups for experiments (in this way, your backups are always of the youngest age possible).
11) Always try to use breeders coming from different pairs (do not pick all your breeders from the same pair, we use at least 4 origins at each generation): that way if a "harmful" mutation occurs, it will not affect all your colony but only a fraction of it, which can be easily reversed. Mutations are rare but can happen naturally: they can involve the transgene (loss of copies, loss or change in expression) or just changes to the genome that result in a problem (physical abnormality, breeding difficulties).
12) We strongly discourage the breeding of an animal presenting a new anatomical or behavioral phenotype (e.g. missing body part, abnormal behavior).
    a. Avoid the inverse by selecting only the best breeders as this can also introduce genetic bias.
13) Keep in mind: 1 pair will give you an average of 2-3 hemizygote (males + females) per month.
14) Keep also in mind that all the statistical information I am giving you are based on breeding at the NIDA IRP facility. If you are obtaining the rats from another source, such as the RRRC, please contact them for updates on breeding.
15) We typically do not cross carrier rats to create "homozygous" lines because the random integration the transgene may have interrupted gene function which is complemented when the carrier is crossed to WT rats.

**Genotyping:**
If working with more than one Cre line at your facility, we recommend using strain specific genotyping protocol rather than a general “Cre PCR” protocol.

For lines with more than one copy, it is essential to monitor copy number as drift may occur over time. We employ droplet digital PCR to monitor copy number.

**Contact:**
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